INHERITANCE OF RESISTANCE TO TOMATO SPOTTED WILT VIRUS IN TOMATOES (*LYCOPERSICON ESCULENTUM* MILL.)

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INTRODUCTION

The tomato, Lycopersicon esculentum Mill, is a leading vegetable crop throughout the world, ranking as the most important commercial vegetable crop not only in the United States but also in many other countries.

Throughout the world, efforts are constantly made to increase total yield per unit area and to produce good quality fruits with all desirable horticultural characters. This requires a constant application and a profound understanding of the principles of crop production, crop improvement, and disease control. The plant breeder is involved in this process in the development of high yielding and disease resistant varieties with superior horticultural characteristics. Diseases are often a major limiting factor in the production of good quality tomatoes, causing reduction in yield as well as quality. Some diseases in some parts of the world completely wipe out the crop and make the cultivation of tomatoes almost impossible without intensive control and prevention practices, which can be so costly that cultivation of tomatoes is no longer profitable. In this kind of situation incorporation of genetic disease resistance is a very important aspect of crop improvement.

An important tomato disease in Hawaii and elsewhere is Spotted Wilt, a very destructive virus disease. This disease is widely distributed in the major tomato growing

areas in Hawaii. Because Spotted Wilt, like other virus diseases, is very difficult to control, tomato cultivation in Hawaii was severely limited until the Hawaii Agricultural Experiment Station developed Pearl Harbor and subsequent cultivars, which were resistant to the disease.

However, it appears that a new strain of the virus has evolved so that the cultivars that were formerly resistant are no longer resistant to the new strain. Thus, it is necessary to develop new resistant cultivars to cope with the evolution of the new strain of the pathogen.

Resistance to this disease has been reported from many places, in particular Hawaii, Australia, Brazil, Peru, and the United Kingdom. Numerous lines have been reported to be resistant but not much has been studied on their genetics. The purpose of this study is to investigate the inheritance of resistance from various sources and to explore the possibility of utilizing such materials in the development of new resistant lines or cultivars.

LITERATURE REVIEW

History and early reports

Brittlebank (1919) first reported this disease in the state of Victoria, Australia, and named it "Spotted Wilt" of tomato. Osborn (1919) also reported the same disease in tomato in South Australia. In 1920 this disease was reported to be prevailing in all the states of Australia (Best, 1968). Samuel et al. (1930) carried out an extensive study of this disease and were the first to associate the disease with a virus, which they named "Tomato Spotted Wilt Virus". By this time the disease was well established in Australia and had become a very serious tomato disease.

K. M. Smith (1931, 1932) was the first scientist to record this disease outside Australia, in the United Kingdom. Subsequently the disease has been reported from many other parts of the world, including Europe, South America, Africa, and Asia. This indicates that the disease is cosmopolitan in distribution.

Moore (1933) reported that the Kromneck disease in South Africa had a probable co-identity with Spotted Wilt in terms of symptomology of the disease and the virus-vector relationship. Gardner et al. (1935) confirmed that the disease was transmissible by Thrips tabaci. Mcwhorter and Milbrath (1939) reported that the Tomato Tip Blight Virus in southern Oregon was transmissible by Thrips tabaci and was

closely related to Tomato Spotted Wilt. Holmes (1948) reported that Ring Spot disease of Dahlia pinnata (Dahlia) was similar to Spotted Wilt of tomato in greenhouse and laboratory tests. He further reported that infected plants of Dahlia could serve as a possible source of inoculum because the virus is capable of overwintering in Dahlia whereas it could not overwinter in tomato grown as an annual crop in New Jersey.

Linford (1932) reported that the pineapple Yellow Spot disease, known in Hawaii ever since 1926, was similar to Spotted Wilt with respect to vector-virus specificity but not in symptomology and host range. He did not conclude that the pineapple Yellow Spot disease was the same as Spotted Wilt disease of tomato. Gardner et al. (1935) stated that the transmission of Tomato Spotted Wilt Virus and pineapple Yellow Spot Virus to Emilia and the symptoms produced on Emilia were identical in Queensland and South Africa, where Tomato Spotted Wilt was well established and the pineapple Yellow Spot disease was also found. Thus it was speculated that the causal agent of Tomato Spotted Wilt and the Yellow Spot disease of pineapple was the same.

Symptomology

The symptomology of this disease has been reported from many areas where the disease infects tomatoes such as Australia (Samuel et al., 1930), California (Gardner et al.,

1935), Brazil (Foster and Costa, 1938), New Zealand (Chamberlain and Tylor, 1938), United Kingdom (K. M. Smith, 1957), and Japan (Kobatake et al., 1976).

The leaf symptoms of the disease can be classified as necrotic and pigmented lesions and patterns, mild surface necrotic ringspot and etch patterns, and yellow and non-necrotic mosaic mottles (Best, 1968). All of these symptoms have been reported in tomato, tobacco (Nicotiana tabacum), Nicotianum glutinosa, and other species. Some plants may exhibit only a part of the above spectrum of symptoms, while others may show all of them (Best, 1968). Samuel et al. (1930) and Smith (1932) described the symptoms in Solanaceous crops, particularly tomato, and suggested that the disease would better be called "bronzy wilt" after the chief characteristics of the disease. The diseased plant first shows slight intensification or thickening of veins of the young leaves, which may be accompanied by one or two concentric rings, and the young leaves may curl slightly inwards and downwards (Figure 1). The next symptom may be the characteristic bronzing of leaves. The bronzing may be in the form of bronze colored circular markings or it may cover the leaf surface completely. At a later stage, the leaves may develop bold yellowish mosaic mottlings.

Milbrath (1939) described additional symptoms for Tomato Tip Blight, the most virulent strain of Spotted Wilt

Virus. These are marked blighting and blackening of the terminal shoot, with black patches and brown streaks on the dead tip. The leaves of affected shoots show a few large or numerous small black necrotic spots on both surfaces; these may enlarge and coalesce. The development of the fruit may be arrested and irregular brown spots may appear on the green surface.

Samuel et al. (1930) and Smith (1932) also described the development and appearance of symptoms on the fruits. They found no symptoms of the disease on fruits set prior to infection but reported pronounced symptoms on fruit set after infection. The symptoms consist of pale red, often yellow, or more rarely white areas in the skin of the ripe tomato (Figure 2). These plane areas may vary in shape from irregular blotches to distinct concentric circles. Sakimura (1940) also reported the same symptoms on tomato.

Symptoms on other plants

(a) Nicotiana tabacum: Lesions develop on inoculated or newly infected leaves of tobacco either as concentric rings with central spots or large plaques with zones of necrotic tissues (Samuel et al., 1930, Smith, 1932).

(b) Solanum capsicastrum (winter cherry): The virus was reported to have been recorded on this plant first outside Australia. The main symptom is ringspot and it has

been reported that ringspot reaches its highest development in this species (Samuel, Bald, and Pittman, 1968).

(c) Spinach: No primary lesions have been reported on this species. Systemic symptoms include grayish brown necrotic spots, marginal wilt and necrotic streaks on petioles, and eventual death of the plants (Samuel, Bald, and Pittman, 1968).

(d) Emilia: Spotted Wilt Virus symptoms at the beginning consist of marked circular ringspots on the leaves (Figure 3). In the later stage a number of brown to black necrotic spots appear on the affected leaves. The terminal portion of the plant becomes twisted and distorted.

Transmission and vector relationship

Tomato Spotted Wilt Virus has been reported to be transmitted by only one family of insects, the Thripidae. Pittman (1927) was the first to report that Thrips tabaci Lind was responsible for the transmission of the virus. Pittman (1927), Samuel et al. (1930) and Bald et al. (1931) claimed that the virus was transmissible by Thrips tabaci Lind and Franklinella insularis Samuel, as well as by mechanical inoculation. Bald and Samuel (1931) first reported that insects belonging to the family Thripidae can only transmit the virus as adults if they had fed on an infected plant as a larva. Bald and Samuel (1931) also reported that an incubation period of several days was

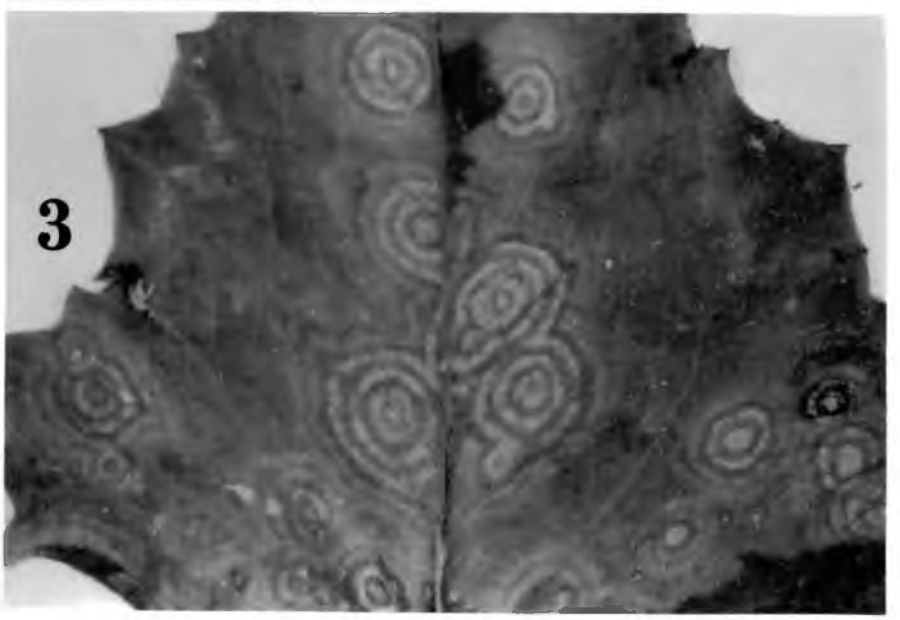
Figure 1. Symptoms of Spotted Wilt Virus on tomato plant: inward and downward curling of leaflets, and stunted growth of plant.

Figure 2. Symptoms of Spotted Wilt Virus on ripe tomato fruit: pale red and yellow areas in the skin of the tomato fruit.


1


2

Figure 3. Spotted Wilt Virus symptom on Emilia sonchofolia: appearance of marked circular ringspots and necrotic spots.


3

required before the virus would be transmitted by the Thrips. The fact that Thrips must feed on infected plant tissues in the larval stage in order to transmit the virus as an adult was confirmed later by Smith (1932) in England and Linford (1932) in Hawaii. The transmission of the virus by species of Thrips has been reported by Moore (1933) in South Africa, and Gardner et al. in Brazil (Best, 1968).

Sakimura (1961) reported that some species of the family Thripidae were misidentified. (For instance, the species identified by Samuel et al. in 1930 as Franklinella insularis should have been identified as F. schultzei). Best (1968) concluded that, even if misidentification was considered, there are at least four species of the family Thripidae that are proven vectors of the Spotted Wilt Virus, Thrips tabaci Lind, Franklinella schultzei Trybon, F. fusca Hinds, and F. occidentalis Pergande. He also reported that there were no significant differences among the four species regarding their efficiency as transmitters of the virus.

Sakimura (1961) observed a 15-minute acquisition threshold period for Thrips tabaci and he also observed a correlation between the percentage of infection and the length of the feeding period; 4% infection after a 15-minute feeding, 33% after 1 hour, 50% after 1 day, and 77% after 4 days. The reasons for the differences between larvae and adults in their ability to acquire and transmit the virus

are not yet known. Best (1968) and Samuel (1936) showed that Tomato Spotted Wilt Virus needs a suitable pH and redox potential for maintaining infectivity in vitro. Day and Irzykiewez (1954) speculated that differences in the pH or redox potential of the midgut of larvae and adults might be the cause of the differences in ability to acquire the virus but a midgut puncturing experiment showed no differences in pH or redox potential between larvae and adults. No other experiments on this have been seen and nothing can be said about how the larvae are able to acquire the virus. It is also not known if the virus multiplies in its insect vector and no experiments have been reported to test this.

Mechanical transmission

The virus is reported to be mechanically transmissible (Samuel et al., 1930, Smith, 1930-32, Samuel, Bald, and Pittman, 1968, Best, 1968) but certain techniques such as use of abrasives and a ground glass spatula greatly enhance the transmission. It has been suggested that a certain amount of reducing agent such as sodium sulphate be added to the inoculum because it stabilizes the virus. It has also been shown that the mechanical transmission could be greatly enhanced if inoculum is prepared in a buffer solution at pH 7.0 because at neutral pH the virus particles are more stable and retain more infectivity.

Samuel et al. (1930) reported that conditioning the plants in the dark for 24 hours before inoculation could increase the total amount of infection. When the inoculum is prepared in a buffer solution at the optimal pH value, the electrolyte concentration is controlled by a reducing agent, abrasive is used on the leaves to be inoculated, and the plants are conditioned for 24 hours in a darkened place before inoculation, it should be possible to obtain a very high percentage of infection, perhaps 100% by mechanical inoculation.

Host range

The host range of tomato Spotted Wilt Virus has been reported to be extremely wide. Best (1968) reported 163 species plants in 34 families hosts with 60 of the 163 species in the Solanaceae. This means Solanaceae is the largest family serving as host to this virus. Some of the important hosts belonging to the Solanaceae are:

Lycopersicon esculentum

Nicotiana tabacum

Nicotiana glutinosa

Solanum capsicastrum

Solanum melongena

Capsicum annuum

Datura stramonium

Hyoscyanum niger

Lycium ferrossium

The general symptoms on these species may be summarized as follows:

Browning of the leaves with circular markings, appearance of concentric rings with central spots, necrotic ringspot, and leaves covered with concentric circles.

Strains

Variability in symptoms of diseases may be attributed to two causes; non genetic factors such as environment and biological factors such as new variants of the causal organism or of the host. When a host plant is grown under a specific environment, the symptoms which develop are regarded as an invariable property of the pathogen in that environment. Thus, differences in symptoms shown by a specific host or by differential hosts in the same environment are the basis on which strains of a pathogen are recognized. There may be a wide range of symptoms produced by different strains of a pathogen in the same species or cultivar of a host plant. Likewise, a given strain of a pathogen may also produce a wide range of symptoms in different species or cultivars of hosts (Matthews, 1970).

According to Finlay (1952) and Best (1968), Norris first reported strains of Spotted Wilt Virus in 1946. These were called Tip Blight (TB), Necrotic (N), Ringspot (R), Mild (VM), and Very Mild (VM). Best and Gallus (1950, 1953)

also worked on strains of the Spotted Wilt Virus. They named six strains A, B, C1, C2, D, and E, with strain A the most virulent and E the most mild. They separated the six strains on the basis of 3 differential hosts; Lycopersicon esculentum (Var. Dwarf Champion), Nicotiana glutinosa, and Nicotiana tabacum. The diagnostic characters of the strains manifested by these differential hosts (as reported by Best, 1968) are listed in Table 1.

Strain A is the only strain that causes apical and stem necrosis on L. esculentum (Var. Dwarf Champion). Strain B causes leaf necrosis on L. esculentum (Var. Dwarf Champion) as well as both N. glutinosa and N. tabacum. Strains C1 and C2 differ from strain B because they produce mottling on L. esculentum. Strain C2 also produces ringspot on N. tabacum although C1 does not. Strain D is distinguished because it is the only one that causes purple pigmentation on L. esculentum. The only symptoms of the mild strain E are mottle on L. esculentum and ringspot on N. tabacum.

Thus, strain A seems similar to the Tip Blight strain of Norris, B the necrotic strain, C1 and C2 perhaps the ringspot, D the mild strain, and E the very mild strain.

Finlay (1952) reported additional work on strains of the Spotted Wilt Virus. He used L. peruvianum, Porter's strain of L. pimpinnelifolium, and the L. esculentum cultivars, Rey de los Tempranos, Manzana, and Pearl Harbor

TABLE 1. -- Symptoms produced by strains of Spotted Wilt Virus on Lycopersicon esculentum (Var. Dwarf Champion), Nicotiana glutinosa, and N. tabacum (Best and Gallus, 1950, 1953)[z]

Strains	Hosts						
	L. esculentum				N. glutinosa	N. tabacum	
	Na	N1	P	M	N1	R	N1
A	+	-	-	-	+	-	-
B	-	+	-	-	+	-	+
C1	-	-	-	+	+	-	+
C2	-	-	-	+	-	+	+
D	-	-	+	-	-	-	+
E	-	-	-	+	-	+	-

[z]Na = Apical necrosis

N1 = Leaf necrosis

P = Purple pigmentation

M = Mottle

R = Ringspot

to differentiate the strains further into 10 different strains which he grouped into four groups according to the symptoms they produced on tomato (Table 2). He reported 3 strains in the Tip Blight group, 2 in the Necrotic group, 3 in the Ringspot group, and 2 in the Mild group, all which were distinguished by the symptoms they produced on 5 different hosts. Three types of reactions are reported by Finlay, immune or no reaction to the virus, resistant in which symptoms are produced but the plant is able to resume growth normally, and susceptible when symptoms are produced and the plant is overcome by the disease.

L. peruvianum was the most resistant, being immune to all but 1 strain and resistant to that. Next was L. pimpinnelifolium which was immune to the Ringspot and Mild groups of strains, and resistant to all the Necrotic and Tip Blight strains except that it was susceptible to strain TB2. Rey de los Tempranos and Pearl Harbor were immune to only the Mild strains, and both were susceptible to at least 1 strain in each of the other groups. Although Manzana was resistant to 4 strains, it was susceptible to at least 1 strain in every group.

Thus, Finlay has added a new dimension to strain identification in Spotted Wilt Virus, distinguishing strains by their ability to infect different host genotypes as well as their ability to produce different symptoms on a specific genotype.

TABLE 2. -- Reaction of differential hosts (tomato) to strains of Spotted Wilt Virus (Finlay, 1952)

Hosts	Tomato Spotted Wilt Virus Strains									
	Tip Blight			Necrotic		Ringspot			Mild	
	TB1	TB2	TB3	N1	N2	R1	R2	R3	M1	M2
L. peruvianum	R[Z]	I	I	I	I	I	I	I	I	I
L. pimpinnelifolium	R	S	R	R	R	I	I	I	I	I
Rey de los Tempranos	R	R	S	S	R	S	R	I	I	I
Pearl Harbor	S	S	R	R	S	R	S	S	I	I
Manzana	S	R	S	S	S	S	R	R	S	R

[Z]I = Immune, which means plants did not show reaction to the virus even after two inoculations.

R = Resistant, which means plants exhibited symptoms, but they were able to overcome the effect of the virus and continue growth free of virus.

S = Susceptible, which means plants exhibited symptoms and were unable to overcome the effect of virus.

Genetic Recombination and the Evolution of New Strains

Best (1968) showed that there can be a transfer of some character determinant (genetic factor) between two strains while they multiply in an infected leaf, and this transfer of genetic material can change the ability to evoke pathogenesis. He postulated that a new strain of virus can arise from recombination because "in mixedly infected plants, there is an exchange or transfer of genetic determinants between the particles of the strains early in the multiplication process as the strains multiply in the tissues." Best and Gallus (1955) verified this theory with an experiment on recombination in tomato and tobacco. Since then recombination has been considered to be a significant factor in the evolution of new strains of Spotted Wilt Virus of tomato.

Control of Spotted Wilt Virus. Control measures of tomato Spotted Wilt Virus can be classified into two catogories, non-genetic and genetic.

(a) Non-genetic Control Measures. Non-genetic control measures include parasitism, control of vectors, good management, and cross protection.

(i) Parasitism. Biological control of the Thrips vector is possible, K. M. Smith (1932) reported two records of Hymenoptenus parasites of species of Thrips, Thripoctenus brui in France attacking Franklinella robusta, and

Thripoctenus russelli in the United States attacking Thrips tabaci. No further work on biological control of Thrips has been reported. However, biological control cannot eliminate the vector population completely, so it is likely that there will always be some insects that are capable of transferring the virus.

(ii) Management Practices. Management practices such as roguing and burning infected plants, eradicating weed and volunteer plants that can serve as a reservoir of vectors and virus, and regulating temperature if possible, can greatly minimize the incidence of infection by Spotted Wilt Virus (Best, 1968). Smith (1932) suggested not growing other host plants in or near a glasshouse in which tomatoes are grown, but this may not always be possible when weeds are difficult to control.

In South Australia tomatoes can be successfully grown in greenhouses without being affected by the virus because the chief vector in South Australia is Franklinella schulzei which does not like the conditions in the greenhouse and does not enter. In Victoria, the neighboring state, however, the chief vector is Thrips tabaci which likes conditions in the greenhouse and readily enters and infects the tomatoes (Best, 1968).

(iii) Cross Protection. Infection of a plant by a mild strain of a pathogen sometimes seems to protect that

plant against damage by a more severe strain of the same pathogen. This effect is called cross protection.

The possibility of cross protection with Spotted Wilt was shown by Best (1968) when he found that plants infected first by the milder strains of the virus, C1, C2, and E, and then by the more severe strains A and B expressed less severe symptoms than plants that were infected only by the severe strains. Thus, he suggested that tomato plants be infected with the mild strain E before being transplanted to the field so they are protected from the severe strains and thus the amount of loss is minimized.

(b) Genetic Control. Breeding for resistance has been widely carried out to cope with diseases in economically important plants, because resistance is often effective in supressing epiphytotics, especially when combined with other means such as control of insect vectors, planting in isolation, and destruction of diseased plants. While the cost of developing a resistant variety is high, it can be less in the long run than the cost of applying other control measures, especially if the resistance remains effective for a long time. This economic consideration has been a major impetus behind breeding programs for disease resistance.

Inheritance Studies Reported. The earliest study reported only that Spotted Wilt resistance and small size fruits were linked (Samuel et al, 1930). The first

inheritance study (Kikuta et al, 1946) reported that the resistance in Pearl Harbor, which originated from Porter's strain of L. pimpinnelifolium, was controlled by a single dominant gene. Holmes (1948), however, working in New Jersey, reported that the resistance in the cultivars, Rey de los Tempranos and Manzana, was controlled by a single recessive gene.

Finlay (1952, 1953) tremendously increased the understanding of the mechanism of inheritance of resistance to Spotted Wilt Virus. He used Porter's strain of L. pimpinnelifolium and three L. esculentum cultivars, Rey de los Tempranos, Manzana, and Pearl Harbor, as the sources of resistance. He crossed each of the four resistant tomatoes with the susceptible cultivar, Potentate, to obtain F_1s and F_2s. He also crossed the four resistant types in all possible combinations to test and determine allelism. The four F_1 hybrids obtained by crossing the four resistant types with the susceptible Potentate showed three levels of resistance when tested with the 10 strains of Spotted Wilt Virus he had identified. Each of the hybrids were fully resistant to some strains, completely susceptible to others, and susceptible but with a delayed systemic infection to still others. The control cultivar Potentate was fully susceptible to all 10 strains of the virus.

In the F_2 segregating populations, Finlay reported that each F_2 plant that was resistant to one strain of a group, was also resistant to other strains of the same group. This, however, was not also true in the F_1, because here the plants often reacted differently to strains within the same group. He concluded that the resistance to each strain group of Spotted Wilt Virus was monogenic. He reported five single genes responsible for resistance to the four strain groups and he suggested that they be designated $SW1^a$, $SW1^b$, sw2, sw3, and sw4. $Sw1^a$ and $SW1^b$ are dominant and allelic and sw2, sw3, and sw4 are independent and recessive.

He further reported that L. pimpinnelifolium and Rey de los Tempranos each possessed four genes for resistance, the former having all but $SW1^b$ and the latter having all but $SW1^a$. Manzana had $SW1^b$ and sw3 and Pearl Harbor had $SW1^a$ and sw4.

Thus, the cross between L. pimpinnelifolium and Rey de los Tempranos should produce about 50% F_2 plants resistant to all 10 strains of the virus because the heterozygous plants having both dominant genes ($SW1^a$ and $SW1^b$) as well as being homozygous for all the recessive genes (sw2, sw3, and sw4) have all possible genes for resistance.

When Finlay (1951) tested the F_1 hybrid between Pearl Harbor and Rey de los Tempranos, he found that it was resistant under field conditions although both parents were

susceptible. This may be because the hybrid received the gene $SW1^a$ from Pearl Harbor and the gene $SW1^b$ from Rey de los Tempranos (just like the case discussed above) which would make the hybrid more resistant than either parent to the tip blight strains of the virus.

MATERIALS AND METHODS

Plant Materials. The plant materials used in this study (Table 3) included 11 PI lines reported to be resistant to Spotted Wilt Virus (Smith, 1942, Holmes, 1948, and Skrdla et al, 1968), two Hawaiian cultivars (Anahu and Kewalo) reported to carry the SW-1 gene for Spotted Wilt Virus resistance (Gilbert, 1956, 1973), one Hawaiian breeding line (8248) and one Florida cultivar (Floradade) with no Spotted Wilt resistance, and a line called Brazil which has been observed to be resistant to TSWV in Hawaii even when Anahu and Kewalo were not (Tanaka, personal communication). The 11 PI lines included one line of L. pimpinnelifolium, eight lines of L. peruvianum, and two lines of L. esculentum. All other materials were L. esculentum except possibly the line called Brazil, which appeared to have some characters of both L. peruvianum and L. esculentum. Crosses were attempted among all the lines, but no crosses with L. peruvianum succeeded. The tomato lines with which successful crosses were obtained, and which were used in the inheritance study, are as follows;

PI 79532 (L. pimpinnelifolium); This is a small cherry type red currant tomato. This line was originally tested for resistance to TSWV in Hawaii by Frazier et al in 1946 and found to be highly resistant. The characteristics of PI 79532 are indeterminate growth habit, small leaflets, small

TABLE 3. -- Lycopersicon lines collected for Spotted Wilt Virus inheritance study.

Lines	Species[Z]	Origin	Source[X]	TSWV reaction reported	Reference
PI 79532	pimp	Peru	NCRPIS	Resistant	Skrdla et al., 1968
PI 203229	escu	Aust.	NCRPIS	Resistant	Holmes, 1948
PI 203230	escu	Aust.	NCRPIS	Resistant	Holmes, 1948
PI 126928	peru	Peru	NCRPIS	Resistant	Smith, 1942
PI 126930	peru	Peru	NCRPIS	Resistant	Smith, 1942
PI 126944	peru	Peru	NCRPIS	Resistant	Smith, 1942
PI 126946	peru	Peru	NCRPIS	Resistant	Smith, 1942
PI 128657	peru	Peru	NCRPIS	Resistant	Smith, 1942
PI 128659	peru	Peru	NCRPIS	Resistant	Smith, 1942
PI 128660	peru	Peru	NCRPIS	Resistant	Smith, 1942
Kewalo	escu	Hawaii	UHSL	Resistant	Gilbert, 1973
Anahu	escu	Hawaii	UHSL	Resistant	Gilbert, 1950, 1971

TABLE 3. (Continued) Lycopersicon lines collected for Spotted Wilt Virus inheritance study.

Lines	Species[Z]	Origin	Source[X]	TSWV reaction reported	Reference
Line 8248	escu	Hawaii	UHSL	Susceptible[Y]	
Floradade	escu	Florida	UHSL	Susceptible[Y]	
Brazil	Unknown	Brazil	Unknown	Resistant[Y]	

Z pimp = L. pimpinnelifolium, escu = L. esculentum, peru = L. peruvianum

Y J. S. Tanaka, personal communication

X NCRPIS = North Central Region Plant Introduction Station, Ames, Iowa

UHSL = University of Hawaii, Seed Laboratory

fruits (.37") with globose shape, medium to heavy fruit set, and medium maturity (Skrdla et al, 1968).

PI 203230 (L. esculentum); This is an Argentinian cultivar called Rey de los Tempranos. It has previously been tested for resistance to TSWV by Finlay (1952) in Australia and by Holmes (1948) in New Jersey and Hawaii. Holmes (1948) reported that F_2 progenies from a cross between Rey de los Tempranos and the susceptible Rutgers segregated in the ratio of 3 diseased to 1 resistant and attributed the resistance in Rey de los Tempranos to a single recessive gene. He also reported that it was less resistant than the Hawaiian cultivar Pearl Harbor in Hawaii, although it was more resistant in New Jersey. Finlay (1952) also tested this cultivar in Australia and found it had a high level of resistance to Spotted Wilt. He preferred to use it as a parent instead of L. pimpinnelifolium because of its superior horticultural qualities. The characteristics of PI 203230 are; indeterminate growth habit, small to medium leaflets, fruit diameter 1.5", fruit shape oblate, fruit set medium, cracking severe, and late maturity (Skrdla et al, 1968).

PI 203229 (L. esculentum); This is another Argentinian cultivar, Manzana. Holmes (1948) tested Manzana in New Jersey for TSWV susceptibility, and reported that some lines were resistant in New Jersey, but in Hawaii, like Rey de los

Tempranos, it was less resistant than Pearl Harbor. Finlay (1952) also tested Manzana in Australia and reported that it possessed a gene which he designated $SW1^b$, which controlled resistance to the Australian Tip Blight Number 2 (TB 2) strain of the Virus. The characteristics of PI 203229 are; indeterminate growth habit, small to medium vine size, medium leaflets, fruit diameter 2", shape oblate, fruit set medium, cracking light, and late maturity (Skrdla et al, 1968).

Anahu (L. esculentum); This cultivar was developed from Pearl Harbor and is supposed to carry the SW-1 gene for resistance to Spotted Wilt in Hawaii (Gilbert, 1952, 1971). The characteristics of Anahu are; determinate growth habit, medium vine size, prolific fruit set, medium maturity, large fruits, and uniform ripening.

Kewalo (L. esculentum); Kewalo was selected from progeny of a cross between a bacterial wilt resistant line of L. pimpinnelifolium and the cultivar Anahu. It was also supposed to carry the SW-1 gene which was incorporated from Anahu (Gilbert, 1973). The characteristics of Kewalo are; determinate growth habit, medium vine size, prolific fruit set, medium maturity, large fruits, and uniform ripening.

Line 8248 (L. esculentum); This is a breeding line developed in Hawaii by Gilbert and Tanaka. The characteristics of line 8248 are determinate growth habit,

compact plant, short internodes, large globe fruits, medium maturity, and uniform ripening. It has not been observed to be resistant to Spotted Wilt Virus (J.S. Tanaka, personal communication).

Floradade (L. esculentum); This cultivar was developed in Florida. It is reported to be resistant to race 1 and 2 of Fusarium Wilt, Verticilium Wilt, and Gray leaf spot (Asgrow Seed Company). The characteristics of Floradade are; determinate growth habit, heavy and vigorous foliage, medium large fruits, jointless stem, and uniform ripening (Asgrow Seed Company). It is not resistant to Spotted Wilt Virus.

Brazil; This line was originally introduced to Hawaii from Brazil by a private individual (K.Y. Takeda, personal communication). Morphologically, Brazil appears most like L. esculentum but also has L. peruvianum characters. The characteristics of Brazil are; semideterminate growth habit, vigorous foliage, prolific fruit set, small fruit size, and uniform ripening.

II) Testing for Resistance

The Tomato Spotted Wilt Virus can be readily transmitted by mechanical inoculation as well as by the vector Thrips tabaci (Lind). In this experiment the test plants were inoculated mechanically as well as exposed to the Thrips vector.

(i) Testing conditions. All testing for resistance was carried out in the greenhouse. Plants to be tested were grown in Speedling trays in a mixture of Vermiculite, Perlite, and Peatmoss in the proportion of 3:1:1. The Speedling trays used were the 32 hole size (8 by 4, 3" x 3" holes) with one plant in each hole. When the seedlings had reached the eight leaf stage at about 4 weeks of age, they were conditioned for mechanical inoculation by placing them in a completely dark place for about 24 hours.

(ii) Maintenance of inoculum. The Spotted Wilt Virus was maintained in the greenhouse on Emilia sonchifolia and on susceptible tomato plants. The Spotted Wilt Virus inoculum was initially obtained from M. Ishii, Plant Pathology department. The Emilia sonchifolia and the susceptible tomato plants were inoculated when they were 3 weeks old. Inoculum was obtained from infected tomato plants 12 to 15 days after infection and reinoculated on new lots of susceptible seedling tomato and Emilia sonchifolia plants. This process was repeated whenever necessary to keep freshly inoculated plants available.

(iii) Mechanical transmission

(a) Inoculum preparation. Materials needed for inoculum preparation are as follows;

.1 M Phosphate buffer (Ph 7.0)

Reducing agent .01 M Sodium metabisulphite

Mortar and Pestle

Abrasive powder and Cheesecloth

Procedure. Spotted Wilt Virus infected Emilia or tomato leaves and shoots inoculated 12 to 15 days previously were added to the buffer and the reducing agent solution in a proportion of roughly one gram plant tissues to one ml buffer solution in a Mortar. A small amount of abrasive was also added to facilitate the entry of the virus particles into the plant cells upon inoculation. The plant tissues were crushed and finely macerated with the Pestal and then filtered through the Cheesecloth.

(b) Inoculation. Tomato seedlings which had been conditioned in the dark for about 24 hours were inoculated by applying inoculum on the third leaf from the top of the seedling. The inoculum was applied with a small soft brush dipped into the inoculum and then gently rubbed on the surface of the leaflets. The inoculum was always applied immediately after its preparation because Spotted Wilt Virus is unstable and loses its infectivity rapidly. After inoculation the Speedling trays containing the inoculated seedlings were placed on the greenhouse benches to be exposed to the Thrips vector.

(iv) Vector transmission. After being mechanically inoculated the tomato seedlings were also exposed to the insect vector Thrips tabaci. Emilia sonchifolia is a

preferred host of this Thrips, so a large population of Thrips developed on the Emilia plants in the greenhouse. Speedling trays with Emilia and Thrips were alternated with Speedling trays with the mechanically inoculated tomato seedlings so that Thrips from the infected Emilia plants would spread to the surrounding tomato seedlings and infect any which had escaped the mechanical inoculation.

(v) Development of symptoms. Observations were started 5 days after inoculation and continued until 55 days after inoculation. The development of symptoms by the plants during this period was as follows;

The first symptoms which appeared as soon as 8 days after inoculation were brown necrotic spots along with general bronzing on the inoculated leaf (Figure 4, 5).

The next symptoms, observed as soon as 12 - 14 days after inoculation, were interveinal and marginal necrosis on other leaves, and inward and downward curling of the leaflets. Stem growth appeared to be checked (Figure 6).

22 - 28 days after inoculation the affected plants started to produce profuse, small, and irregular bronze colored leaflets around the tip of the shoot. The shoot itself became somewhat twisted and abnormal in appearance. The infected plants appeared stunted. The terminal leaflets showed necrotic spots and lesions beginning on the tip and proceeding toward the center, eventually resulting in total

necrosis of the leaflets(Figure 7, 8). The green shoots would gradually turn brown to dark brown to black and die. These symptoms are called Tip Blight. Most plants died soon after Tip Blight occurred but in some plants the lower portion remained alive even after severe necrosis and death of the shot occurred.

It took about 50 days after inoculation for complete development of disease symptoms in tomato plants grown in greenhouse.

(vi) Disease classification. The disease reactions shown by the tomato seedlings up to 55 days after inoculation were classified into 5 different classes. Each plant was scored individually and placed in one of the following classes.

Class 1. Plants that showed no disease symptoms whatsoever. Plants in this class looked healthy and normal and were apparently free of disease (Figure 9).

Class 2. Plants in this class showed light yellowing of a few leaves, a few necrotic spots, and a slight tendency of the leaflets to curl inward and downward (Figure 10).

Class 3. Plants in this class displayed severe necrosis on the leaflets and shoots, and appeared stunted (Figure 11).

Class 4. Plants in this class showed shoot death (severe necrosis and eventual death of the shoot), but the lower portion of the plant remained alive (Figure 12).

Class 5. Plants in this class had died (shoot death accompanied by the whole plant death, Figure 13, 14).

III) Crossing Procedure

Tomato flowers are complete with a functioning androecium (anthers) and gynoecium (pistil). Flowers are in clusters with individual flowers borne on peduncles. The flower cluster develops on an elongated peduncle. Each flower cluster usually consists of 4-8 flowers. The tomato is highly self pollinated with pollination usually occurring soon after the first pollen is released from the anther. Anthers are removed from the flowers that are to be pollinated before any pollen has been released. The right time for emasculation is when the green sepals start separating to expose the upper part of the anthers, which should then be changing from green to yellow. Emasculating and pollinating at the same time, usually the day before the flower would open, is generally practiced to save time and labor. Pollen is obtained from a flower in which the anthers have just dehisced. The pollen is collected by stripping the anther cone with a flattened or spearpoint needle. The same instrument can be used to transfer the pollen to the stigma of the female parent. All crosses were made in the greenhouse. The first anthesis occurred approximately 55 days after planting. All pollinations were made during the next 2 weeks.

Figure 4. General yellowing and bronzing of infected tomato leaflets.

Figure 5. Mosaic mottlings on the infected tomato leaflets.


4


5

Figure 6. Inward and downward curling of leaflets, appearance of Tip Blight on shoot, and checked shoot growth.

Figure 7. Total shoot necrosis: the green shoot turned to dark brown to black.

Figure 8. Death of the terminal shoot (final stage of Tip Blight).

6

7

8

Figure 9. Healthy tomato plant, rated as class

Figure 10. Yellowing of leaves and appearance of brown necrotic spots. The plant was rated as class 2.


9


10

Figure 11. Necrosis on leaves and shoot. Stunted plant growth. The plant was rated as class 4.

Figure 12. The plant showed shoot death but lower portion of the plant remained alive. The plant was rated as class 4.

Figure 13. Shoot death accompanied by plant death. The plant was rated as class 5.


11


12


13

Figure 14. Spotted Wilt Virus symptoms produced by susceptible check (Floradade): severe shoot necrosis and eventual death of the plants.


14

IV) Inheritance Studies

F_1 seeds were planted in Speedling trays in the greenhouse and then transplanted to the Waimanalo Research Station to obtain seeds for F_2 plants. F_2 seeds along with parents, and F_1's were planted in the greenhouse in the first week of July, 1982 for inheritance studies. The seedlings were inoculated in the first week of August, 1982 when they reached the eight leaf stage at 4 weeks of age. They were evaluated for their resistance to TSWV in the last week of September, 1982. 55 days after inoculation, the F_2 data were analyzed and tested by the Chi-square goodness of fit test.

Two resistant plants from the cross between line 8248 and Brazil were backcrossed to both the parents. One of the plants used for backcrossing was an F_1 plant of the original cross made by J.S. Tanaka. The other plant used was an F_3 plant produced by selfing an F_2 plant which was resistant to Spotted Wilt.

RESULTS

Crosses which produced seeds. There were a total of 7 crosses which produced hybrid seeds (Table 4). 4 L. esculentum lines (PI 203229, Anahu, Floradade, and line 8248) were crossed with Brazil and 3 L. esculentum lines (PI 203230, Kewalo, and Floradade) were crossed with PI 79532 (L. pimpinnelifolium).

Crosses which did not produce hybrid seeds (fruit set without seeds). In two crosses (PI 203230 X PI 126946, and Floradade X PI 126946) in which PI 126946 (L. peruvianum) was used as a male parent, only seedless fruits were obtained. This is probably the "incongruity" described by Hogenboom (1973). He found that in crosses between L. esculentum and L. peruvianum, the peruvianum pollen often stimulates fruit development but there is only sub-normal or no embryo development resulting in seedless fruit.

Failure of fruit set. Some crosses did not set any fruit (Table 5). Two of the crosses that did not set fruit, even though a total of 10 flowers were pollinated over a period of 3 days, involved the line Brazil. The lineage of this line is unknown. It has some characters of L. peruvianum, but it crosses readily with some L. esculentum lines, which L. peruvianum does not usually do. It did not cross with Kewalo, which is L. esculentum or with PI 79532, which is L. pimpinnelifolium. Perhaps there is some residual

TABLE 4. -- Crosses which resulted in hybrid seed

Female parent	Species	Male parent	Species
Floradade	L. esculentum	PI 79532	L. pimpinnelifolium
Floradade	L. esculentum	Brazil	Unknown
Line 8248	L. esculentum	Brazil	Unknown
Anahu	L. esculentum	Brazil	Unknown
Kewalo	L. esculentum	PI 79532	L. pimpinnelifolium
PI 203229	L. esculentum	Brazil	Unknown
PI 203230	L. esculentum	PI 79532	L. pimpinnelifolium

TABLE 5. -- Crosses that did not set fruit

Female parent	Species	Male parent	Species
Brazil	Unknown	PI 79532	L. pimpinnelifolium
Kewalo	L. esculentum	Brazil	Unknown
Anahu	L. esculentum	PI 79532	L. pimpinnelifolium

incompatibility as a result of some L. peruvianum genes which may be causing this lack of fruit set. The other cross which did not set fruit was Anahu X PI 79532. Both of these parents produced seeds when crossed with other parents and there is no expected incompatibility between them, so it is not possible to explain the lack of fruit set in this cross.

Reaction of parents to Spotted Wilt Virus Infection

The reactions of the parental lines to inoculation by Spotted Wilt Virus are presented in Table 6. The mean infection for the different lines ranged from 4.45 (most susceptible) to 1.93 (most resistant) with a clear division into 2 groups. Floradade, line 8248, Anahu, and Kewalo were susceptible with mean disease ratings ranging from 4.45 to 4.02, while PI 203229, PI 203230, PI 79532, and Brazil were resistant with mean disease ratings ranging from 2.25 to 1.93. The resistant line with the lowest disease rating (most resistant) was Brazil. This confirms the resistance observed by Tanaka (personal communication). PI 79532 (L. pimpinnelifolium) had the next lowest disease rating. Several L. pimpinnelifolium accessions have been reported to have Spotted Wilt Virus resistance (Kikuta et al., 1946, Finlay, 1952, and Skrdla et al., 1968) and one was involved in the parentage of Pearl Harbor, the first Spotted Wilt Virus resistant cultivar developed in Hawaii (Kikuta et al.,

TABLE 6. -- Classification of tomato lines for Spotted Wilt Virus Infection

Lines	Infection class[Z] and number of plants					Total	Disease Rating	
	1	2	3	4	5		Mean[X]	Variance
Floradade			6	10	24	40	4.45[a]	.5625
Line 8248			6	19	23	48	4.35[a]	.4889
Anahu			8	19	13	40	4.13[a]	.5224
Kewalo			11	23	12	46	4.02[a]	.5106
PI 203229	5	36	9			50	2.25[b]	.5636
PI 203230	4	28	11			43	2.16[b]	.3300
PI 79532	3	43	7			50	2.02[b]	.1420
Brazil	10	29	7			46	1.93[b]	.3734

[Z]Class 1 is most resistant and the class 5 is most susceptible

[X]Means followed by the same letter are not significantly different from each other (Duncan's Multiple Range Test)

1946). Pearl Harbor received its Spotted Wilt resistance from a line of L. pimpinnelifolium in its parentage which was originally received in Hawaii from Gardner in California who had received it from Porter (Kikuta et al, 1946). Frazier et al (1946) tested PI 79532 for resistance to Spotted Wilt in Hawaii but do not say whether this is the same line as that used by Porter. However, Gilbert (personal communication) has reported that PI 79532 was the original source of resistance to Spotted Wilt Virus in Hawaii. Finlay (1952) in Australia again refers to "Porter's strain of L. pimpinnelifolium" as a source of resistance to this virus. Thus, although none of the published reports specifically identify PI 79532 as being Porter's strain of L. pimpinnelifolium, it is possible and may be even probable that they are the same. PI 203230 and PI 203229 have also been tested previously in Hawaii but under the names of Rey de los Tempranos and Manzana (Holmes, 1948). Holmes reported that both cultivars were less resistant than Pearl Harbor in Hawaii although they were more resistant in New Jersey. Pearl Harbor is no longer available and could not be tested in this study, but these two lines were not quite as resistant as the most resistant Brazil and PI 79532, although the difference was not significant.

Anahu was developed from Pearl Harbor and has been reported to have the SW-1 gene for resistance to Spotted

Wilt (Gilbert, 1956). Kewalo was developed from Anahu and has also been reported to be resistant to Spotted Wilt (Gilbert, 1973). However, neither of these cultivars are now resistant (Table 6). There could be two possible explanations for this. Either these cultivars have lost the resistant genes they once possessed or a new strain of Spotted Wilt Virus could have appeared. It does appear, however, that Anahu and Kewalo show slightly lower disease ratings than Floradade and line 8248, which are the most susceptible. Perhaps, Anahu and Kewalo still have some genes for resistance, but they are not as effective as those in the really resistant cultivars.

F_1 GENERATION RESULTS

The Spotted Wilt reactions of the F_1 populations ranged from 2.28 to 1.94 (Table 7), all of which are similar to the resistant parents (2.25 to 1.93). The variances ranged from .7341 to .2641, which were also similar to the parental variances. The highest means (2.28 to 2.24) were observed in the crosses between one susceptible parent (Floridade or line 8248) and one resistant parent (Brazil or PI 79532). The next highest means (2.10 and 2.04) were observed in the crosses in which Anahu or Kewalo (susceptible) were crossed with Brazil or PI 79532 (resistant). The lowest means (1.96 and 1.94) were observed in the two crosses in which both parents were resistant (PI 203230 X PI 79532 and PI 203229 X

TABLE 7. -- Classification of F_1 plants for Spotted Wilt Virus Infection

Crosses	Infection class[Z] and number of plants					Total	Disease Rating	
	1	2	3	4	5		Mean	Variance
Floradade X Brazil	5	23	6	5		39	2.28	.7341
Line 8248 X Brazil	3	28	8	3		42	2.26	.4920
Floradade X PI 79532	4	25	5	4		38	2.24	.6181
Anahu X Brazil	4	20	7			31	2.10	.3570
Kewalo X PI 79532	5	34	7			46	2.04	.2646
PI 203230 X PI 79532	8	35	6			49	1.96	.2900
PI 203229 X Brazil	9	35	6			50	1.94	.3024

[Z]Class 1 is most resistant and class 5 is most susceptible

Brazil). The high level of resistance of the F_1 populations suggests that resistance is dominant.

All F_1's were confirmed to be hybrids by their morphological characters. All the F_1's which had Brazil as a parent looked like Brazil with semi-indeterminate habit, somewhat flat fruits, and fruit size similar to but a little bigger than Brazil. The F_1's which had PI 79532 as a parent all looked like L. pimpinnelifolium with indeterminate habit and cherry type fruits.

F_2 GENERATION RESULTS

The results of testing the F_2 plants for their reaction to Spotted Wilt are presented in Table 8. All F_2 populations segregated into 5 disease classes with the greatest number of plants in class 2. The means ranged from 2.54 to 2.21.

The highest F_2 means (2.54 to 2.50) were observed in the three crosses between one susceptible and one resistant parent (Floridade X PI 79532, line 8248 X Brazil, and Floradade X Brazil). The next highest means (2.44 and 2.42) were observed in the two crosses between the Hawaiian cultivars and one of the resistant parents (Kewalo X PI 79532, and Anahu X Brazil). The lowest means (2.27 and 2.21) were observed in the two crosses in which both parents were resistant (PI 203230 X PI 79532, and PI 203229 X Brazil). Although these means are not significantly

TABLE 8. -- Classification of F_2 plants for Spotted Wilt Virus Infection

Crosses	Infection class[Z] and number of plants					Total	Disease Rating	
	1	2	3	4	5		Mean	Variance
Floradade X PI 79532	21	144	19	23	25	232	2.54	1.24
Line 8248 X Brazil	13	87	14	12	16	142	2.52	1.29
Floradade X Brazil	18	108	16	14	20	176	2.50	1.30
Kewalo X PI 79532	38	122	16	25	23	223	2.44	1.41
Anahu X Brazil	37	125	17	24	22	225	2.42	1.38
PI 203230 X PI 79532	64	91	28	34	10	227	2.27	1.33
PI 203229 X Brazil	43	58	21	20	6	148	2.21	1.44

[Z]Class 1 is most resistant and class 5 is the most susceptible

different it is interesting to note that they follow exactly the same order as the F_1 means (Table 7). The variances ranged from 1.24 to 1.44 which were all considerably higher than the variances in the F_1's as would be expected when genetic segregation occurs in the F_2. On the basis of the similar F_1 and F_2 means and their similar parentage, the F_2 progenies were divided into 3 groups of crosses which behaved similarly, as follows:

Group 1. This group includes the three progenies from the crosses between one resistant and one susceptible cultivar (Table 9). (Examples of this group are illustrated in Figures 15, 16, and 17). The characteristics of this group are about 10% of the F_2 plants were in class 1, about the same as in class 3 and 4, there were more F_2 plants in class 5 than 4; and there were some F_1 plants in class 4. The F_2 means in this group were the highest, meaning that the progenies in this group were the most susceptible.

Group 2. This group includes two progenies from crosses between one of the Hawaiian cultivars and one of the resistant parents (Table 10). (Examples of this group are illustrated in Figures 18 and 19). In this group more than 10% of the F_2 plants were in class 1, class 3 and 4 had considerably less plants than class 1, class 5 had about the same number of plants as class 4, and there were no class 4 individuals in the F_1's. The F_2 means were lower than those

TABLE 9. -- Comparison of Spotted Wilt Virus classifications of group 1 parents, F_1's, and F_2's

Population	Infection class[Z] and number of plants					Total	Disease Rating	
	1	2	3	4	5		Mean	Variance
Floradade			6	10	24	40	4.45	.5615
Brazil	10	29	7			46	1.93	.3734
F_1	5	23	6	5		39	2.28	.7341
F_2	18	108	16	14	20	176	2.50	1.30
8248			6	19	23	48	4.34	.49
Brazil	10	29	7			46	1.93	.37
F_1	3	28	8	3		42	2.26	.49
F_2	13	87	14	12	16	142	2.52	1.29
Floradade			6	10	24	40	4.45	.5615
PI 79532	3	43	4			50	2.02	.1424
F_1	4	25	5	4		38	2.24	.6181
F_2	21	144	19	23	25	230	2.54	1.24

[Z]Class 1 is the most resistant and class 5 is most susceptible

TABLE 10. -- Comparison of Spotted Wilt Virus classification of group 2 parents, F_1's, and F_2's

Population	Infection class[Z] and number of plants					Total	Disease Rating	
	1	2	3	4	5		Mean	Variance
Anahu			8	19	13	40	4.13	.5221
Brazil	10	29	7			46	1.93	.3734
F_1	4	27	7			31	2.10	.3570
F_2	37	125	17	24	22	225	2.42	1.38
Kewalo			11	23	12	46	4.02	.5106
PI 79532	3	43	4			50	2.02	.1424
F_1	5	34	7			46	2.04	.2664
F_2	38	122	16	14	20	223	2.44	1.41

[Z]Class 1 is most resistant and class 5 is most susceptible

TABLE 11. -- Comparison of Spotted Wilt Virus classification of group 3 parents, F_1's and F_2's

Population	Infection class[Z] and number of plants					Total	Disease Rating	
	1	2	3	4	5		Mean	Variance
PI 203229	5	36	9	5		55	2.25	.56
Brazil	10	29	7			46	1.93	.37
F_1	9	35	6			50	1.94	.30
F_2	43	58	21	20	6	148	2.21	1.44
PI 203230	4	28	11			43	2.16	.33
PI 79532	3	43	4			50	2.02	.14
F_1	8	35	6			49	1.96	.29
F_2	64	91	28	34	10	227	2.27	1.33

[Z]Class 1 is most resistant and class 5 is most susceptible

in Group 1, indicating that these progenies were more resistant.

Group 3. This group includes the two progenies from crosses between two resistant parents (Table 11). (Examples of this group are illustrated in Figures 20 and 21). In this group, more than 25% of the F_2 individuals were in class 1 and classes 3 and 4 each had about 3 times as many individuals as class 5. The F_2 progenies in this group were the most resistant.

Testing F_2 Segregation Ratios

Since the F_1's were all resistant and previous reports have suggested that the resistance was due to a single dominant gene, the F_2 data were first tested for a fit to a 3:1 (single gene) ratio by combining classes 1 and 2 as resistant and classes 3, 4, and 5 as susceptible (Table 12). All progenies except PI 203230 X PI 79532 (Group 3) gave a satisfactory fit to a 3:1 ratio. However, Group 3 crosses cannot be segregating for only one pair of genes because although both parents were resistant, susceptible types segregated in the F_2. This can only happen if the two resistant parents have different genes for resistance. Therefore, the F_2 data were next tested for a fit to a two gene 9:3:3:1 ratio (Table 13). The 5 classes were made into 4 by combining classes 1 and 2, but keeping the other classes intact. Although none of the crosses fit a 9:3:3:1

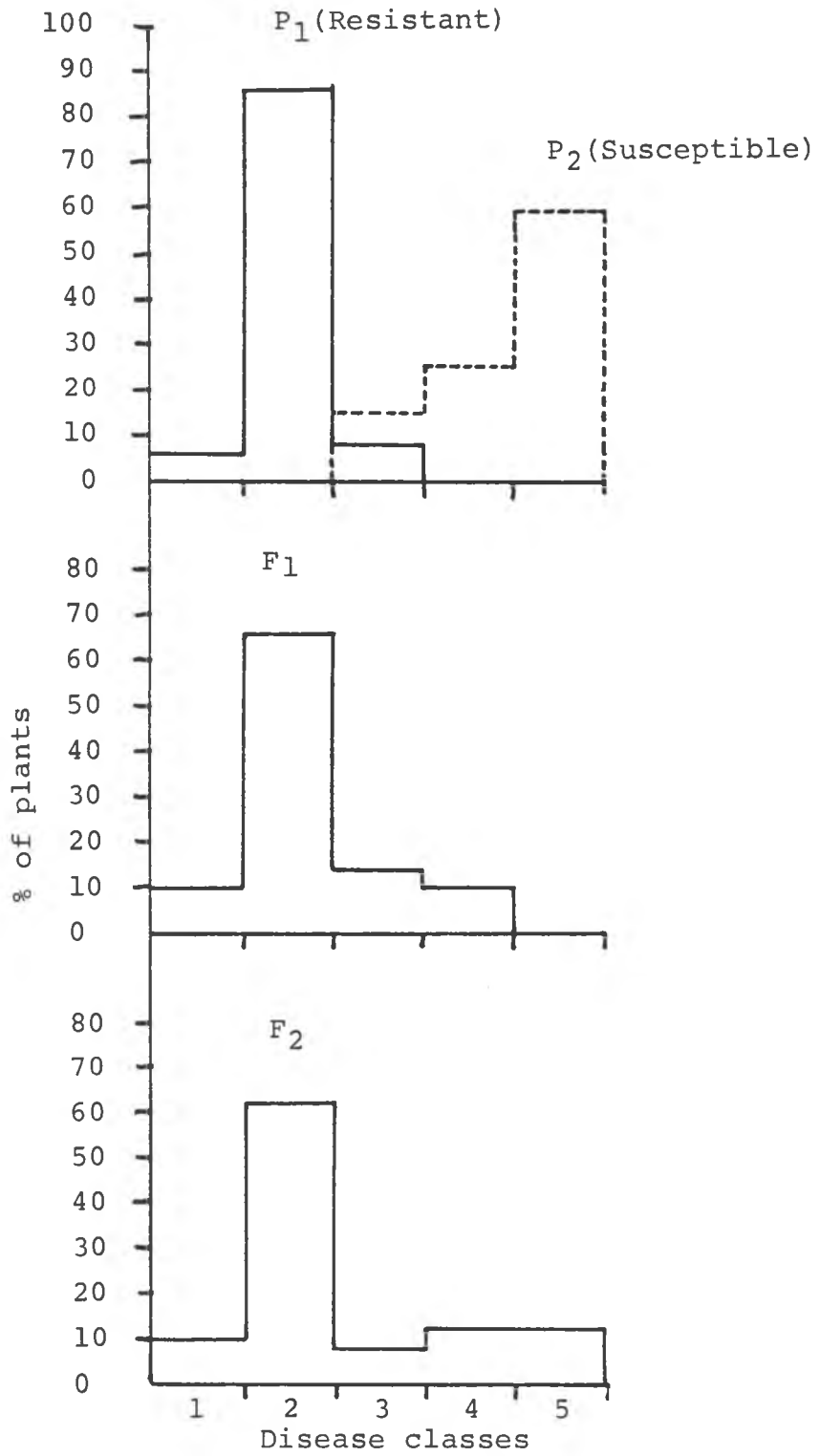


Figure 15; Distribution of Parental, F_1, and F_2 populations from a cross between PI 79532 (P_1) and Floradade (P_2)

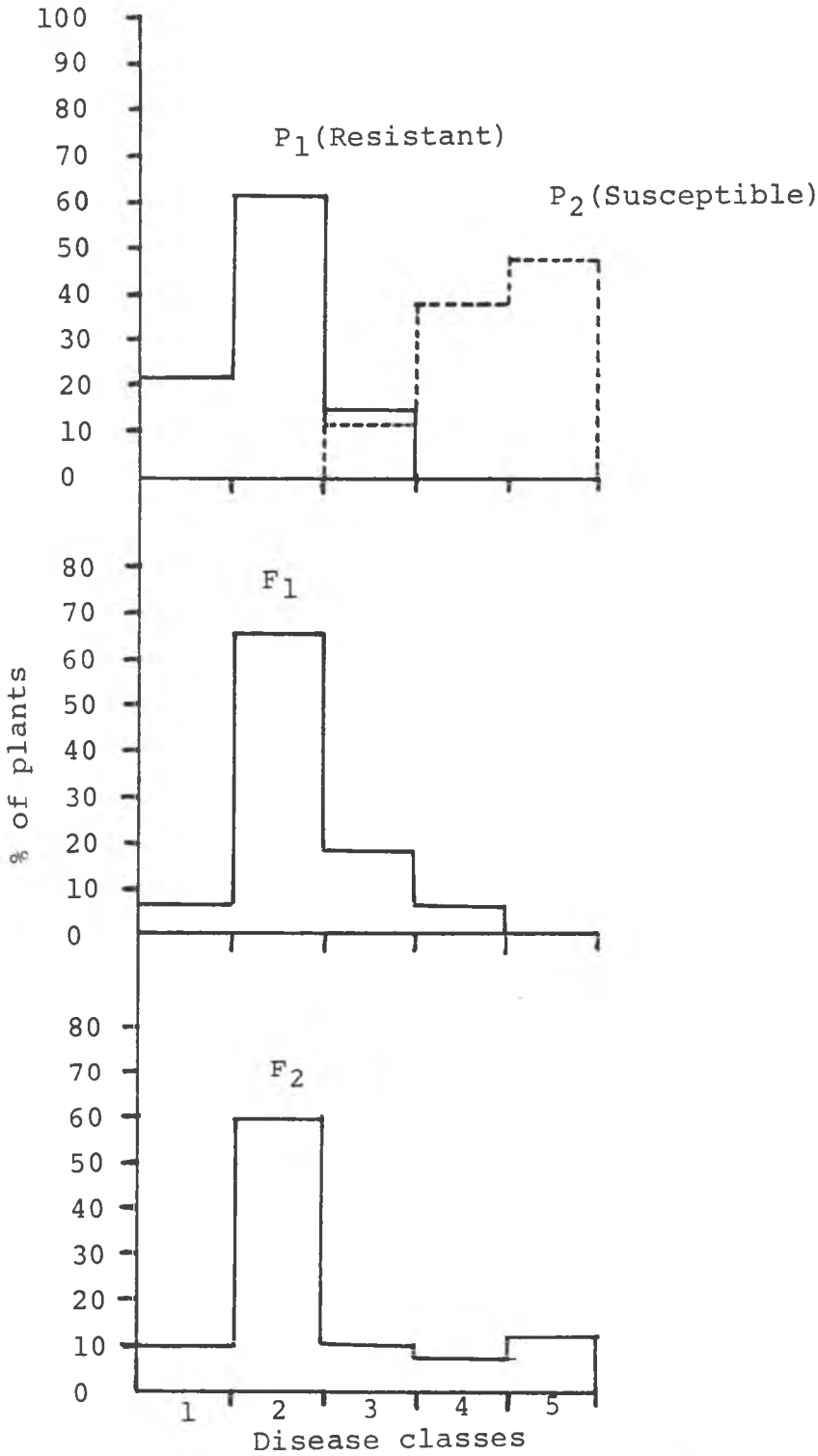


Figure 16; Distribution of parental, F_1, and F_2 populations from a cross between line 8248 (P_2) and Brazil (P_1).

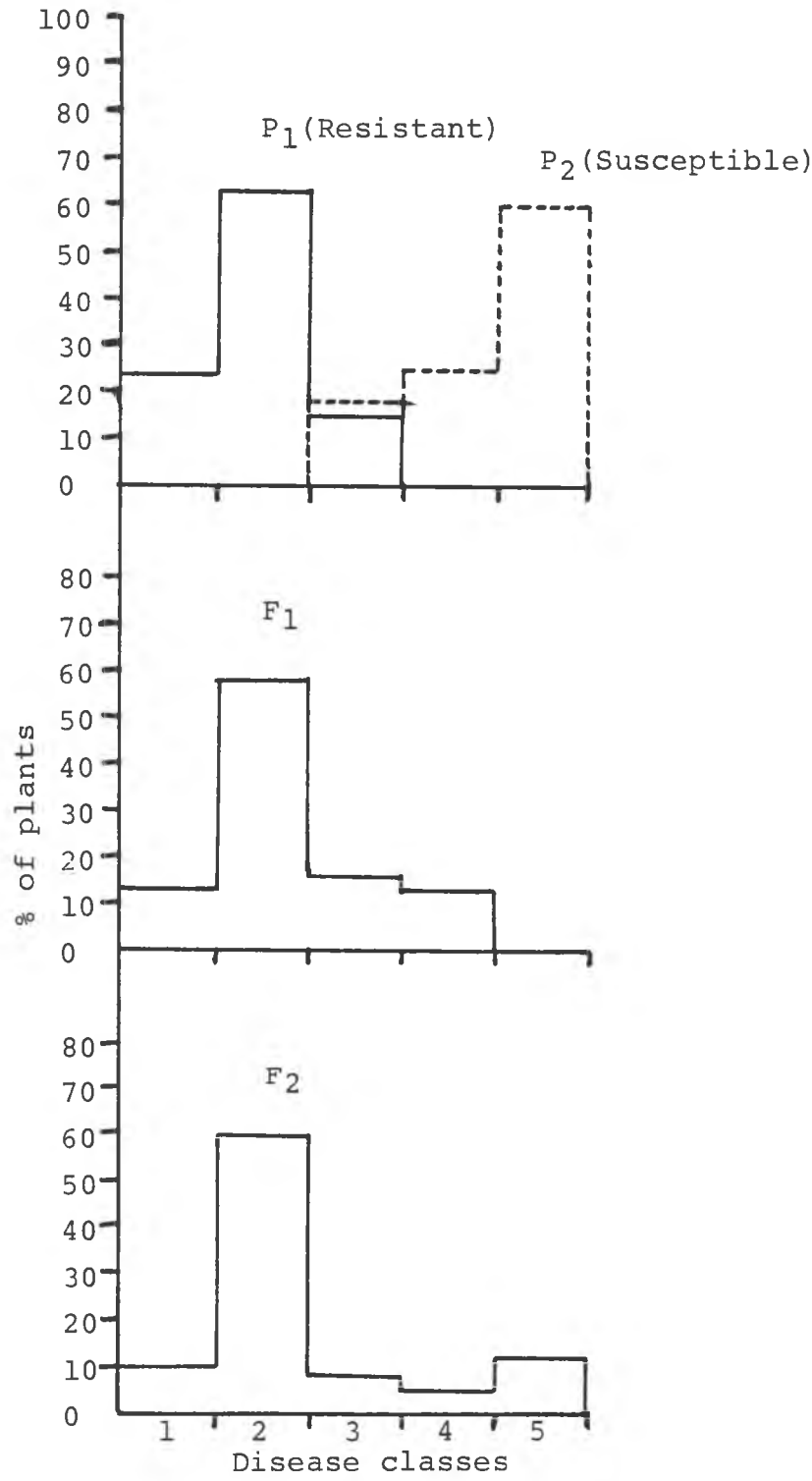


Figure 17; Distribution of parental, F_1, and F_2 populations from a cross between Floradade (P_2) and Brazil (P_1).

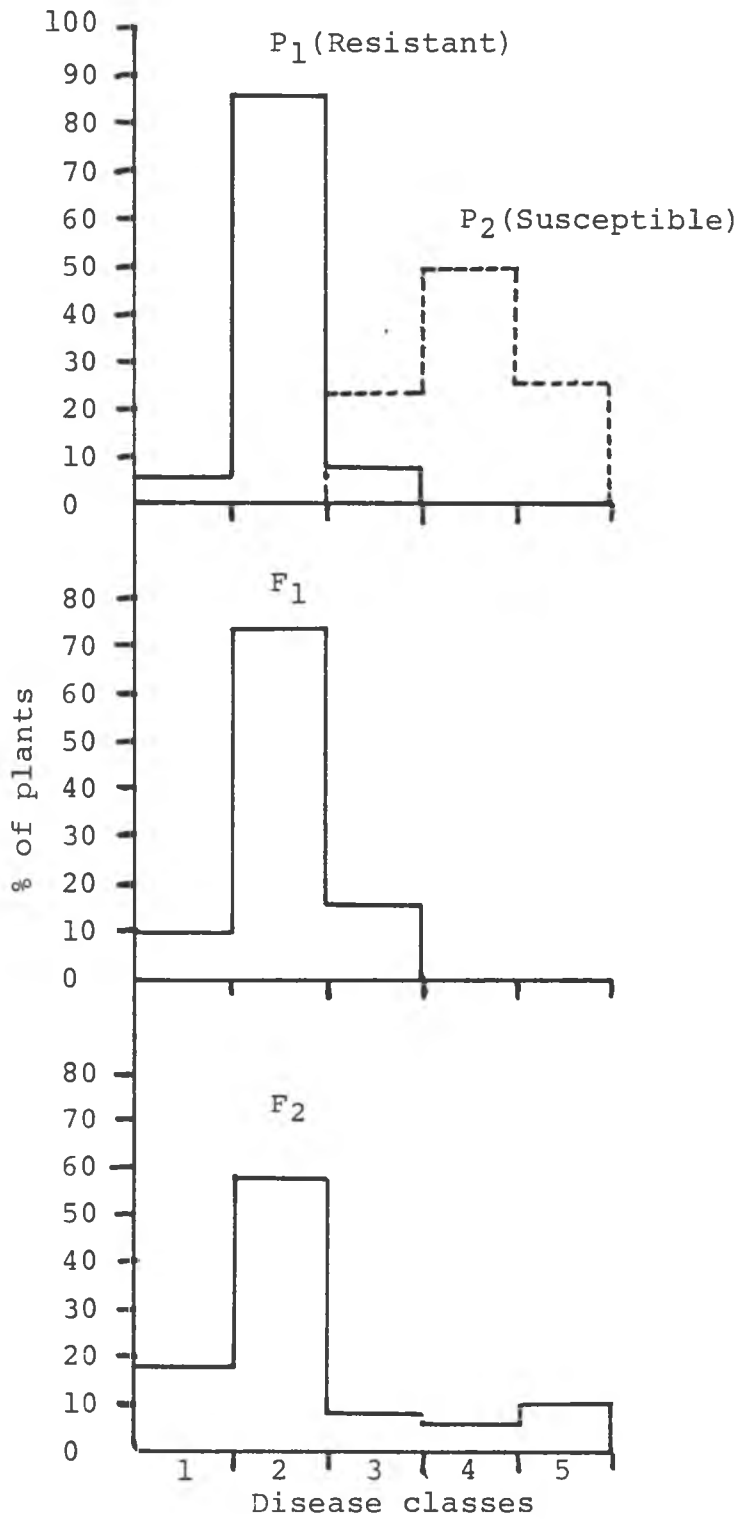


Figure 18; Distribution of parental, F_1, and F_2 population from a cross between Kewalo (P_2) and PI 79532 (P_2).

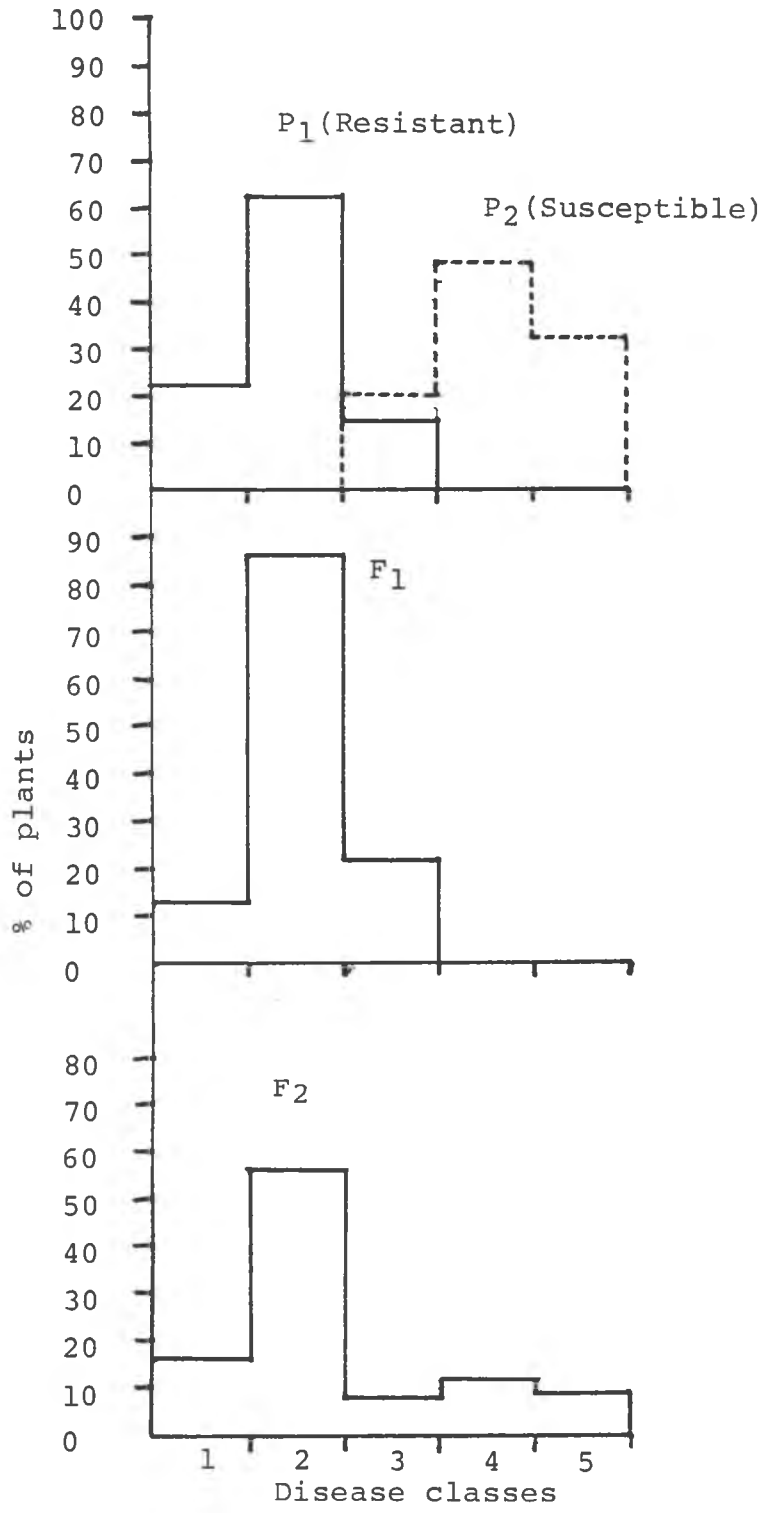


Figure 19; Distribution of parental, F_1, and F_2 populations from a cross between Anahu (P_2) and Brazil (P_1).

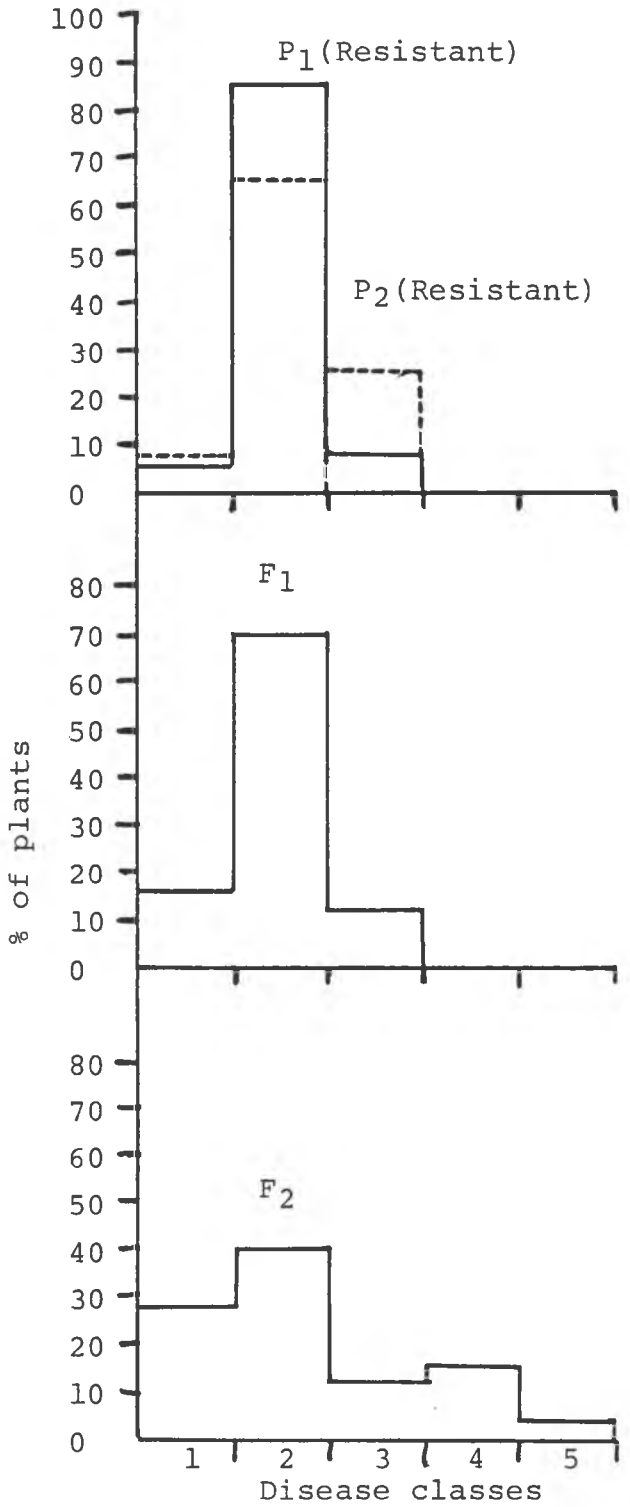


Figure 20; Distribution of parental, F_1, and F_2 populations from a cross between PI 203230 (P_2) and PI 79532 (P_2).

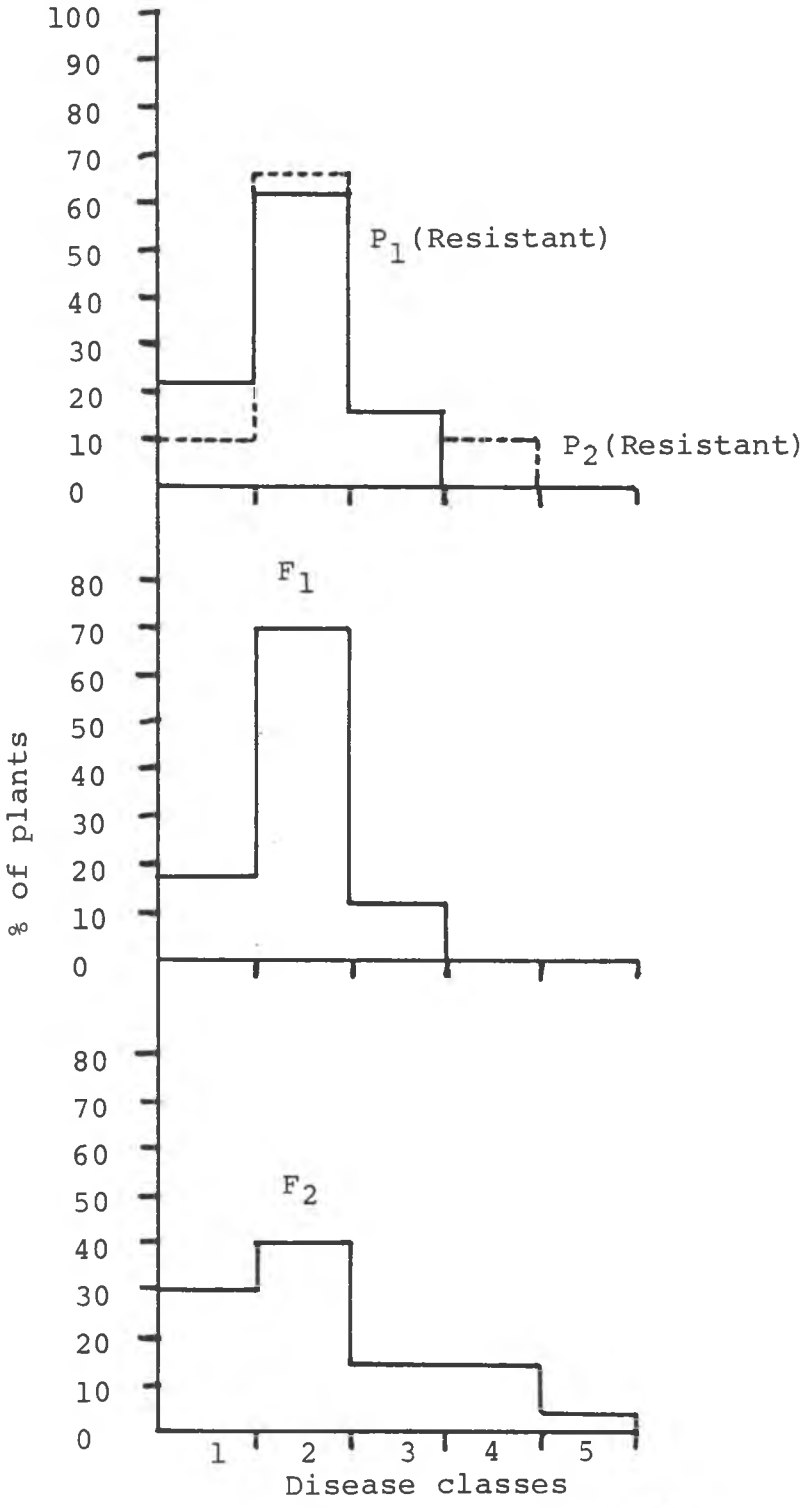


Figure 21; Distribution of parental, F_1, and F_2 populations from a cross between PI 203229 (P_2) and Brazil (P_1).

TABLE 12. -- Testing F_2 segregating ratios for a fit to a 3:1 ratio

Crosses	Infection class and number of plants		Total	Chi-square[Z]
	Resistant (Class 1, 2)	Susceptible (Class 3, 4, 5)		3:1
Group 1				
Floradade X PI 79532	165	67	232	1.3^{NS}
Line 8248 X Brazil	100	42	142	1.5^{NS}
Floradade X Brazil	126	50	176	1.1^{NS}
Group 2				
Kewalo X PI 79532	160	64	224	1.2^{NS}
Anahu X Brazil	162	63	225	1.1^{NS}
Group 3				
PI 203230 X PI 79532	155	72	227	5.7*
PI 203229 X Brazil	101	47	148	3.6^{NS}

[Z]Chi-square values for 1df at 5 and 1% level are 3.841 and 6.635

TABLE 13. -- Testing F_2 segregating ratios for a fit to a 9:3:3:1 ratio

Crosses	Infection class and number of plants				Total	Chi-square[Z]
	(Class 1-2)	3	4	5		9:3:3:1
Group 1						
Floradade X PI 79532	165	19	23	25	232	40.5**
Line 8248 X Brazil	100	14	12	16	142	24.7**
Floradade X Brazil	126	16	14	20	176	49.2**
Group 2						
Kewalo X PI 79532	160	16	25	23	224	38.1**
Anahu X Brazil	162	17	24	22	225	37.3**
Group 3						
PI 203230 X PI 79532	155	28	34	10	227	13.8**
PI 203229 X Brazil	101	21	20	6	148	8.7*

[Z]Chi-square values for 3df at 5 and 1% level are 7.815 and 11.345

ratio, the Chi-square values for the Group 3 crosses were the smallest, suggesting that this Group might have 2 loci segregating.

Since the Group 3 crosses did not fit a 9:3:3:1 ratio, other possible ratios were examined. It was noted that the frequency of class 1 individuals was roughly 25%, which suggested that one pair of genes may distinguish class 1 from the other classes. Likewise, it was noted that classes 2, 3, 4, and 5 occurred in almost exactly a 9:3:3:1 ratio. If a recessive gene which is epistatic and 2 dominant genes are segregating at the same time, the result would be 5 classes in a 16:27:9:9:3 ratio. The F_2 segregations were tested for a fit to this ratio (Table 14). Both Group 3 segregations fit this ratio very well, but, as expected, the Group 1 and Group 2 segregations did not fit this ratio.

Although the Group 2 crosses fit a 3 resistant (class 1 and 2) to 1 susceptible (classes 3, 4, and 5) ratio like Group 1, there seemed to be differences between the two Groups which might be due to genetic causes. Since recessive genes for resistance have been reported (Finlay, 1952, and Holmes, 1948) and the Hawaiian lines seem to have a small amount of resistance, the possibility of a weak recessive gene for resistance was examined. If Group 2 families were segregating for one dominant and one recessive gene for resistance, then the ratio expected would be 3:9:1:3. If classes 4 and 5 are combined the Group 2

TABLE 14. -- Testing F_2 segregating ratios for a fit to a 16:27:9:9:3 ratio

Crosses	Infection class[Z] and number of plants					Total	Chi-square
	1	2	3	4	5		16:27:9:9:3
Group 1							
Floradade X PI 79532	21	144	19	23	25	232	72.8**
Line 8248 X Brazil	13	87	14	12	16	142	44.6**
Floradade X Brazil	18	108	16	14	20	176	54.4**
Group 2							
Kewalo X PI 79532	38	122	16	25	23	224	38.0**
Anahu X Brazil	37	125	17	24	22	225	37.2**
Group 3							
PI 203230 X PI 79532	64	91	28	34	10	227	1.8[NS]
PI 203229 X Brazil	43	58	21	20	6	148	1.4[NS]

[Z]Chi-square values for 4df at 5 and 1% level are 9.488 and 13.27

segregations give a good fit to this 3:9:1:3 ratio, but the other Groups do not (Table 15).

Back crosses

Back crosses to both the resistant parent, Brazil, and the susceptible parent, line 8248, were made with the F_1 of this cross and with a selected resistant F_3 family (Table 16). It can be seen that segregations of the backcross progeny in the two different generations were almost identical. If classes 1 and 2 were combined as resistant and classes 3, 4, and 5 as susceptible, the backcrosses to the susceptible parent gave the expected 1:1 ratio. The backcrosses to the resistant parent, however, had a few individuals in class 3, which is considered to be susceptible. Although it is possible to give a genetic explanation for this, it is more likely that there is a slight spillover of the resistant genotypes into class 3, as was also found for the Brazil parent (Table 16). Therefore, the backcross results seem to support the hypothesis that Brazil and line 8248 differ by one dominant gene for resistance, as was postulated from the F_2 segregation.

TABLE 15. -- Testing F_2 segregating ratios for a fit to a 3:9:1:3 ratio

Crosses	Infection class[Z] and number of plants				Total	Chi-square
	1	2	3	(4 & 5)		3:9:1:3
Group 1						
Floradade X PI 79532	21	144	19	48	232	15.0**
Line 8248 X Brazil	13	87	14	28	142	10.6*
Floradade X Brazil	18	108	16	34	176	9.8*
Group 2						
Kewalo X PI 79532	38	122	16	48	224	1.3^{NS}
Anahu X Brazil	37	125	17	46	225	1.6^{NS}
Group 3						
PI 203230 X PI 79532	64	91	28	44	227	34.2**
PI 203229 X Brazil	43	58	21	26	148	31.9**

[Z]Chi-square values for 3df at 5 and 1% level are 7.815 and 11.345

TABLE 16. -- Testing backcross progenies for a fit to a 1:1 ratio

Crosses	Infection class and number of plants		Total	Chi-square
	Resistant (Class 1 & 2)	Susceptible (Class 3, 4 & 5)		
Line 8248 X Brazil (F_1)	31	11	42	
Line 8248 X Brazil (F_2)	100	42	142	
F_1 X Brazil	111	14	125	
F_3 X Brazil	81	11	92	
F_1 X Line 8248	48	59	107	1.131^{NS}
F_3 X Line 8248	63	75	138	1.034^{NS}

[z]Chi-square values for 1df at 5 and 1% level are 3.841 and 6.625
Note: The classes 1 and 2 were combined as resistant and the classes 3, 4, and 5 as susceptible and tested against 1:1 ratio

DISCUSSION

Although it is likely that the PI 79532 parent used here is either the same or at least carries the same genes for resistance to Spotted Wilt Virus as Porter's strain of L. pimpinnelifolium, which was tested by Finlay (1952) as well as Kikuta et al. (1946), the results here do not agree with those of Finlay (1952). Finlay reported that Porter's strain and Rey de los Tempranos both had alleles of the same SW-1 locus, which is the only locus he reported with a dominant gene for resistance. My results show that PI 79532 and PI 203230 (Rey de los Tempranos) and PI 203229 (Manzana) and Brazil must differ by at least two independent pairs of genes because susceptible individuals segregate in the F_2 after the crosses between two resistant parents. Finlay also did not apparently detect the major recessive gene for resistance in Rey de los Tempranos and Manzana which I found and was reported by Holmes (1948).

In Group 2, which included the two Hawaiian cultivars which have been developed from lines tracing back to the original Pearl Harbor and which have been described as resistant in the past, there does seem to be some residual resistance, perhaps conferred by a pair of recessive genes with only a small effect. Since Finlay (1952) had reported that Pearl Harbor carried one dominant and one recessive gene, it seems possible that Anahu and Kewalo also carried

these two genes formerly when they were considered resistant, but have lost them sometime, or, alternatively, they always had only the one recessive gene, but it is no longer as effective as it formerly was. The reaction of PI 79532 seems to now be just as effective as it was when tested by Frazier in 1946, which would suggest that there have not been any significant changes in the virulence of the virus during that time. Holmes (1948) tested Rey de los Tempranos (PI 203230) and Manzana (PI 203229) for Spotted Wilt Virus resistance in New Jersey and attributed the resistance in these cultivars to a single recessive gene. Finlay (1952) also used these cultivars along with Porter's strain of L. pimpinnelifolium and the Hawaiian cultivar Pearl Harbor in his study of inheritance of resistance to Spotted Wilt Virus in Australia. He reported 4 genes for resistance; one dominant and three recessive. Porter's strain of L. pimpinnelifolium carried the dominant $SW\text{-}1^a$ gene plus the recessive sw2, sw3, and sw4 genes, while Rey de los Tempranos carried the dominant $SW\text{-}1^b$ gene plus the same recessive genes. Manzana carried the dominant $SW\text{-}1^b$ gene plus only the recessive sw3 gene. Pearl Harbor carried the dominant $SW\text{-}1^a$ gene plus the recessive sw4 gene. Finlay based his conclusions on the reactions of the hosts to different strains of the virus as well as on F_2 segregation data. This study appears to confirm a major

recessive gene for resistance in Rey de los Tempranos and Manzana as reported by Holmes (1948) and suggests major dominant as well as some less efficient recessive genes in the L. pimpinnelifolium line (PI 79532) as well as in Brazil.

On the basis of this genetic background information of the parentage involved, a scheme was developed to explain the various gene actions as indicated by Chi-square tests. The probable genetic schemes as determined by the Chi-square values are summarized in Table 17. As can be seen, the Group 1 crosses fit only the 3:1 ratio of those tested. Group 2 crosses fit either a 3:1 ratio or 3:9:1:3 ratio, which actually is a further breakdown of the 3:1 ratio. The Group 3 crosses fit quite well a 16:27:9:9:3 ratio which could be produced by the segregation of 1 locus which shows recessive epistasis and 2 different loci at which resistance is dominant.

The genetic explanation therefore, is as follows:

Group 1 is segregating for 1 pair of genes with resistance dominant. The source of resistance is the resistant parents, PI 79532 or Brazil.

Group 2 is segregating for 1 pair of genes with resistance dominant plus one pair with resistance recessive. The dominant gene has come from the resistant parents, PI 79532 or Brazil, but the recessive gene comes from the

TABLE 17. -- Summary of Chi-square values for group 1, 2, and 3 crosses

Crosses	3:1	9:3:3:1	3:9:1:3	16:27:9:9:3
Group 1				
Floradade X PI 79532	1.3^{NS}	40.5**	15.0**	72.8**
Line 8248 X Brazil	1.5^{NS}	24.7**	10.6*	44.6**
Floradade X Brazil	1.1^{NS}	49.2**	9.9*	54.4**
Group 2				
Kewalo X PI 79532	1.2^{NS}	38.1**	1.3^{NS}	38.0**
Anahu X Brazil	1.1^{NS}	37.3**	1.6^{NS}	37.2**
Group 3				
PI 203230 X PI 79532	5.7*	13.8*	34.2**	1.8^{NS}
PI 203229 X Brazil	3.6^{NS}	8.7*	31.9**	1.4^{NS}

susceptible (but very slightly resistant) parents, Anahu or Kewalo.

Group 3 is segregating for one pair of genes with resistance recessive but epistatic to 2 other pairs of genes at which resistance is dominant. Since PI 79532 and Brazil have been shown to have just 1 pair of genes for resistance at which resistance is dominant, the second pair of genes with resistance dominant and the recessive gene must have come from the other parents, PI 203229 and PI 203230 (Manzana and Rey de los Tempranos).

The scheme postulated, the genotypes of all the parents, the genes segregating in the various crosses, and the F_2 ratios thought to have been observed, are summarized in Figure 22.

Resistant parents	Group 3	Resistant parents
PI 203229 PI 203230		PI 79532 Brazil
Genes involved aaBBccDD	X	AAbbCCDD
(B and c resistant)	F_1	(Only gene A resistant)
Assuming a recessive epistatic effect on dominant genes.	AaBbCc F_2	
	9 A-B-cc*	Disease class
	3 A-bbcc*	
	3 aaB-cc*	1
	1 aabbcc	
* indicates epistatic effects on dominant genes.	27 A-B-C-	2
	9 A-bbC-	3
	9 aaB-C-	4
	3 aabbC-	5

Susceptible parents	Group 2	Resistant parents
Anahu Kewalo		PI 79532 Brazil
Genes involved aabbCCdd	X	AAbbCCDD
	F_1 AaDd	
	F_2	
		Disease class
Gene d is recessive resistant.	3 A-dd	1
	9 A-D-	2
	1 aadd	3
	3aaD-	4&5

Susceptible parents	Group 1	Resistant parents
Floradade		PI 79532
Line 8248		Brazil
Genes involved aabbCCDD	X	AAbbCCDD
	F_1 Aa	
(No resistant genes)	F_2	Disease class
	3^2A-	1&2
	1 aa	3, 4,&5

Figure 22 A DIAGRAMATIC PRESENTATION OF THE SCHEME FOR VARIOUS GENETIC RATIOS.

SUMMARY

The essential points with respect to the mode of inheritance and the gene actions which have stemmed from this study are summarized as follows:

(i) A significant and clear difference was observed between resistant and susceptible parents.

(ii) F_1's of all crosses were equal to the resistant parents with respect to their response to disease reaction, indicating dominance of resistance.

(iii) Segregation was observed in all F_2's, suggesting that the parents involved were genetically different, including the cases of crosses between two resistant parents.

(iv) Group 1 (Susceptible X resistant) crosses fit a 3:1 ratio, suggesting that a single dominant gene is involved. Backcrosses with line 8248 and Brazil confirmed this conclusion. This may be the Sw-1 gene.

(v) The Group 2 (susceptible Hawaiian lines X resistant) crosses fit either a 3:1 or 3:9:1:3 ratio suggesting that the Hawaiian lines (Anahu and Kewalo) may possess a pair of recessive genes conferring a low level of resistance. This may be the sw4 gene reported in Pearl Harbor by Finlay (1952).

(vi) The Group 3 (resistant X resistant) crosses fit a 16:27:9:9:3 ratio, which can result if the parents differed by 3 genes; 2 dominant and 1 recessive and epistatic to the other two. PI 203230 and PI 203229 must have one of the dominant and the recessive epistatic gene since PI 79532 and Brazil have been shown in the other groups to have one dominant gene. These genes seem to be different from those reported by Finlay for these lines.

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